ANTICANCER DRUGS

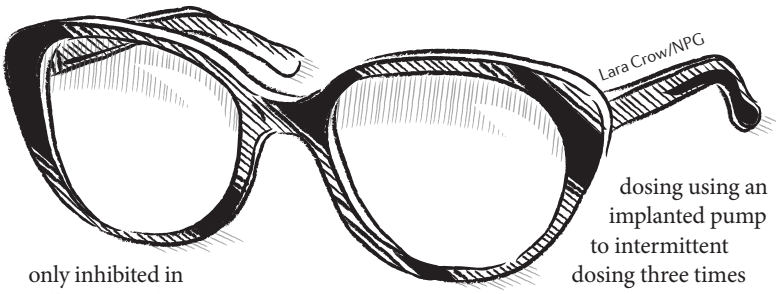
A clearer pathway view

> PI3K functions upstream of RAS to induce rapid apoptosis

Inhibitors of many components of the phosphoinositide 3-kinase (PI3K)–AKT–mammalian target of rapamycin (mTOR) signalling pathway are in various stages of development against different tumour types. However, the success of these inhibitors so far has been minimal, possibly owing in part to a relief of negative feedback inhibition (resulting in reactivation of receptor signalling). To better understand how this pathway functions, Neal Rosen, Sarat Chandarlapaty and colleagues examined the differences between PI3K and AKT inhibitors.

The treatment of BT-474 breast tumour cells (which have amplified ERBB2 and mutated PI3K, and which depend on AKT for proliferation) with the selective AKT inhibitor MK2206 and the selective class I PI3K inhibitor BAY 80–6946 resulted in approximately equal inhibition of AKT and its substrates; however, the inhibition of PI3K induced much more apoptosis. Similar results were observed in two other breast cancer cell lines with amplified *ERBB2*. Analysis of several downstream targets and receptors that might be reactivated by a relief of feedback inhibition led to the discovery that the inhibition of PI3K with BAY 80–6946 inhibited extracellular signal-regulated kinase (ERK) phosphorylation. This was not isolated to BAY 80–6946 or BT-474 cells, as this effect was also observed with several other PI3K inhibitors and in different cell lines from various tumour types.

The inhibition of PI3K also reduced the phosphorylation of MAPK/ERK kinase (MEK) and CRAF, and ERK phosphorylation was only inhibited in cells with wild-type RAS, which suggests that PI3K might directly affect the activation of RAS. Indeed, the levels of active GTP-bound RAS (all isoforms) were reduced in several RAS-wild-type cell lines after PI3K inhibition but not after AKT inhibition.



Further experiments indicated that the inhibition of ERK was required for the induction of apoptosis, and this could be achieved by either PI3K inhibition or the combination of AKT (MK2206) and MEK (PD0325901) inhibition. The authors also noted that although ERK phosphorylation is rapidly inhibited after the inhibition of PI3K, phosphorylated ERK levels are only transiently repressed. This transient inhibition is sufficient to induce apoptosis, although greater levels of apoptosis were induced when ERK inhibition was more sustained (achieved by combining PD0325901 with BAY 80–6946).

BAY 80–6946 also inhibited ERK phosphorylation and induced apoptosis in BT-474 xenografts in immunocompromised mice, as well as in MDA-MB-361 xenografts (which also have amplified *ERBB2* and mutated PI3K). The half-life of BAY 80–6946 *in vivo* is short, so the authors compared continuous dosing using an implanted pump to intermittent dosing three times per week (pulsatile administration). Pulsatile administration more effectively induced apoptosis and suppressed tumour growth than continuous dosing. Similar to the *in vitro* data, adding PD0325901 (administered five times per week) to pulsatile administration of BAY 80–6946 resulted in greater tumour regression, which suggests that a longer ERK inhibition might be beneficial.

That PI3K functions upstream of RAS to induce rapid apoptosis is a key finding in this paper and should help us to better understand these signalling pathways and how to therapeutically target them. In addition, these data suggest that continuous inhibition of these signalling pathways (which often results in severe toxicities) might not be essential to induce antitumour effects and that PI3K inhibitors might be more effective than AKT inhibitors.

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